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Location: **ASRS**

Journal Title: **Annales de technologie agricole.**

Volume: **24** Issue: **3-4**

Month/Year: **1975**

Pages: **397-410**

Article Author: **S. Nemoto**

Article Title: **Possibilities of utilization of butyric acid for rum making**

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POSSIBILITIES OF UTILIZATION OF BUTYRIC ACID BACTERIA FOR RUM MAKING

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SUMMARY

Two methods of production of heavy rum by utilizing butyric acid bacteria have been ...ied.

Butyric acid bacteria could not ferment symbiotically with rum yeast *Shizosaccharomyces* ... in molasses medium containing more than 14 p. 100 glucose.

Addition of 15-30 p. 100 of high-acid mash fermented by butyric acid bacteria to rum mash ... effective for increasing acid content and enriching rum flavor, but ester content increased ... a little.

Distillation of fermented rum mash including high-acid mash at low pH, adjusted to pH = 2, ... available. Acid content increased 4-6 times and ester content 2 times compared with distillate ...non-adjusted, higher pH mash. Lusson Giraid number of fine distillate from non-treated mash ...35, while from adjusted mash is 384.

By lowering pH of mash, butyric acid is let free, and during distillation with alcohol, they ...bine to form ester.

From analytical results, the distillate seemed to be good quality as heavy rum, but it is neces-... to confirm by tasting after aging for a long time if this distillate becomes a good rum or not.

INTRODUCTION

The production and consumption of alcohol in Japan have reached a consi-...rable amount. In the past, domestic sweet potato was mainly used as a raw mate-...l, but now, imported molasses and crude alcohol take the place of sweet potato. ...ble 1, shows alcohol production and its raw materials during the recent four years ... Japan. Sugar cane is not cultivated in Japan, except Okinawa islands, the sou-...ern part of the country. Because of these conditions, it is difficult to get fresh ...olasses. Furthermore, Japanese does not drink rum so much.

TABLE I

Alcohol production and its raw material in Japan

	Year	1971		1972		1973		1974	
	Alcohol production	(kl) 249,494	(%) 100	(kl) 259,041	(%) 100	(kl) 271,110	(%) 100	(kl) 260,718	(%) 100
Raw materials	Molasses	114,850 (368,441 t)	46.0	112,224 (360,015 t)	43.3	104,551 (314,448 t)	38.6	105,780 339,342 t)	40.6
	Hightest Molasses	58,711 (137,223 t)	23.5	55,043 (128,649 t)	21.2	11,550 (26,998 t)	4.3	62 (144 t)	0
	Alcohol Crude			21,969 (25,486 kl)	8.5	92,669 (107,505 kl)	34.2	113,374 (131,374 kl)	43.5
	Others	75,933	30.5	69,805	27.0	62,340	22.9	41,502	15.9

( ) : Material in ton or kl.
From the *Bulletin of Japan Molasses Association*, 1972-1975.

TABLE 2

Production of rum in Japan (1954-1973)

Year	Production (kl)	Year	Production (kl)
1954	10.8	1964	369.3
1955	15.8	1965	376.8
1956	31.5	1966	502.8
1957	66.2	1967	675.2
1958	117.2	1968	735.3
1959	119.0	1969	765.6
1960	134.0	1970	794.1
1961	175.1	1971	839.5
1962	270.3	1972	1 012.0
1963	278.1	1973	1 071.0

From the wine, *Spirits and provisons Monthly Statistics*, 1955-1974.

After the Second World War, goods for daily use were insufficient and their qualities were poor. Materials of confectionary, such as butter and wheat flour, were not an exception. Heavy rum was used in order to improve the taste and flavor of cake made from those bad materials. Thus, rum has been developed not as a drink, but as a confectionary use, and in Japan heavy rum was wanted. Production of rum in Japan (1954-1973) is shown in table 2. We had to study rum making under very different condition from West India.

According to ARROYO (1942), acid forming bacteria, such as acetic and butyric acid bacteria, play an important roll for making heavy type rum. We studied to utilize butyric acid bacteria to make rum containing a large amount of acids and esters. Two methods to utilize butyric acid bacteria, are thought.

1. Rum mash is fermented symbiotically with rum yeast and butyric acid bacteria to form a large quantity of acid and ester ;

2. High-acid mash acidified by butyric acid bacteria is added to rum mash and fermented with rum yeast, and then distillated.

This paper deals with these two methods and their results are reported.

MATERIALS AND METHODS

1. — *Microorganisms*

Butyric acid bacteria : *Cl. butyricum* ATCC-6015 was selected out of 4 strains.
Yeast : *Shizosaccharomyces pombe*. This yeast is used in usual production of our rum factory.

2. — *Composition of medium*

Molasses	3 p. 100 as glucose
$(NH_4)^2SO_4$	0.15 p. 100
KH^2PO_4	0.2 p. 100
$CaCO^3$	0.1 p. 100
pH	6.0-6.2

Composition of medium was decided after some experiments.

3. — *Cultivation condition of butyric acid bacteria*

Cultivation temperature : 30°C.
Incubation of seed culture : 24 hours.

4. — *Analysis*

Analysis was carried out by methods of A.O.A.C.

5. — *Culture method of butyric acid bacteria*

Sand culture of *Cl. butyricum* ATCC-6015 was added to sterilized medium in a 10 ml test tube. It was incubtated for 24 h in anaerobic condition as shown on figure 1.

The contents of the tube were used to inocule 70-80 ml of medium in 100 ml erlenmeyer flask. This was incubated for 24 h and provided inoculum for 700-800 ml of sterilized mash of the same composition. After incubating 24 h, this culture was used for the experiments.

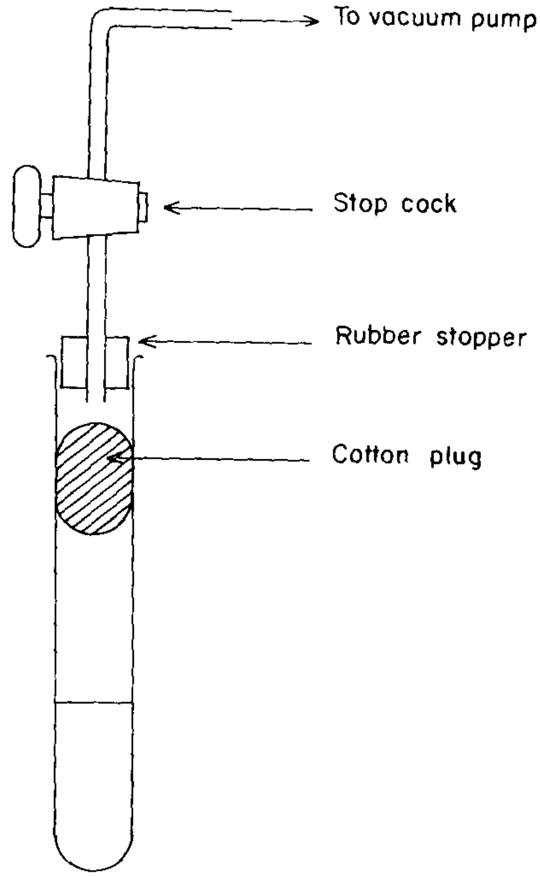

FIG. 1. — *Culture method of butyric acid bacteria*

6. — *Gas chromatography* (Hitachi K-53 gas)

Chromatograph was used. Experimental parameters were as follows.

Column : 2 m ; liquid phase : PEG 1,500 5 p. 100.
Support : Chromosorb W 60/80 mesh.
Column temp. : isothermal 50°C.
Inject temp. : 120°C. Carrier gas : N^2 0.5 kg/cm².
Detector : FID ; H^2 gas flow : 0.6 kg/cm².
Air flow : 2 kg/cm² ; sensitivity : × 20.
Chart speed : 20 mm/mn.

RESULTS

I. — *Influence of sugar and alcohol concentration on butyric acid fermentation*

Rum mash contains comparatively high concentration of sugar before fermentation, and alcohol after fermentation. So, it is necessary to examine if butyric acid bacteria could ferment and form volatile acid in such a medium as rum mash.

1. *Influence of sugar concentration on butyric acid fermentation.*

At first, the influence of sugar concentration on the formation of acid was examined. Sugar concentration of molasses media was adjusted from 2.5 to 15.3 p. 100, to which, seed culture of butyric acid was inoculated and volatile acid was determined after 7 days.

As shown in figure 2, optimum sugar concentration was about 5 p. 100, then acid was decreased and could not be formed at sugar concentration of 15.3 p. 100.

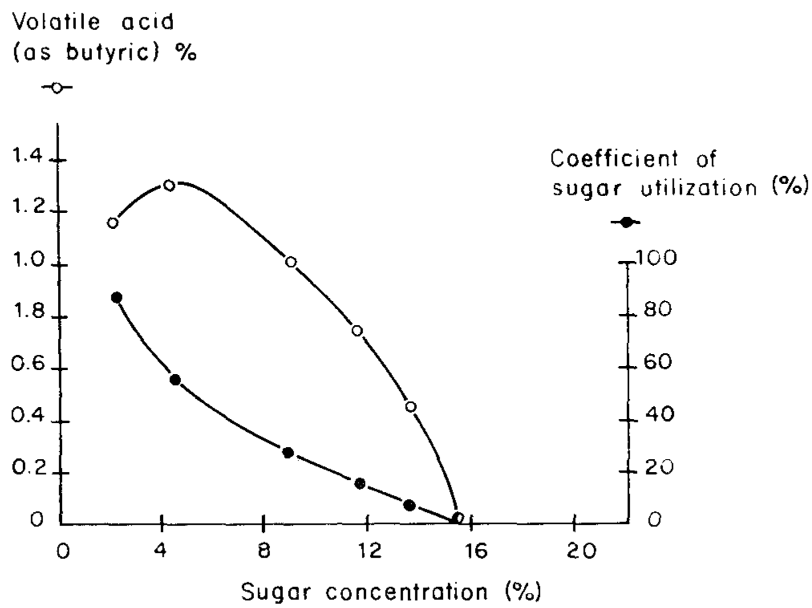

FIG. 2. — *Influence of sugar concentration of butyric acid fermentation*

2. *Influence of alcohol concentration on butyric acid fermentation.*

To examine the influence of alcohol, molasses media containing alcohol from 2.5 to 10 p. 100 were prepared by adding alcohol to molasses media. Volatile acid was determined after incubation bacteria for 7 days.

As shown in figure 3, alcohol strongly inhibited acid formation. Acid was decreased as alcohol concentration increased, and could not be formed at 7.5 p. 100 alcohol.

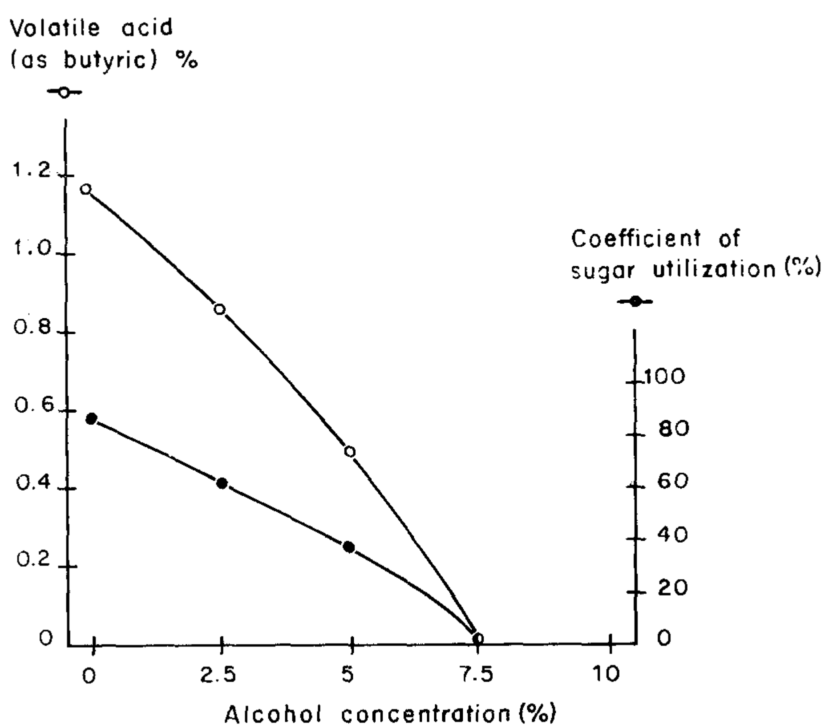

FIG. 3. — *Influence of alcohol concentration on butyric acid fermentation*

3. *Influence of sugar and alcohol concentration on butyric acid fermentation.*

When both sugar and alcohol were contained, the influence of those constituents appeared more strongly. The results of the experiment are shown in figure 4. Volatile acid could not be formed at 5 p. 100 alcohol in the media of 6.9 and 10.5 p. 100 sugar, while in the medium of 2.8 p. 100 sugar, considerable quantity of acid was formed at 5 p. 100 alcohol.

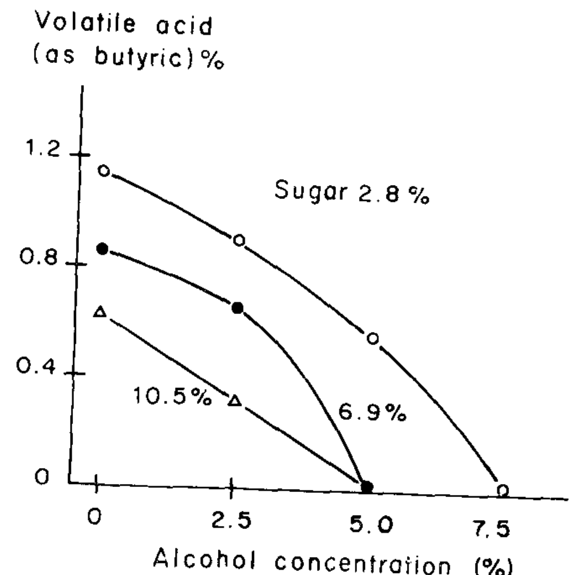

FIG. 4. — *Influence of sugar and alcohol concentration on butyric acid fermentation*

4. *Fermentable range of butyric acid bacteria under various alcohol and sugar concentration.*

Results of above experiments, are shown in figure 5. The inside of curve 1 is the range of sugar and alcohol concentration that butyric acid bacteria

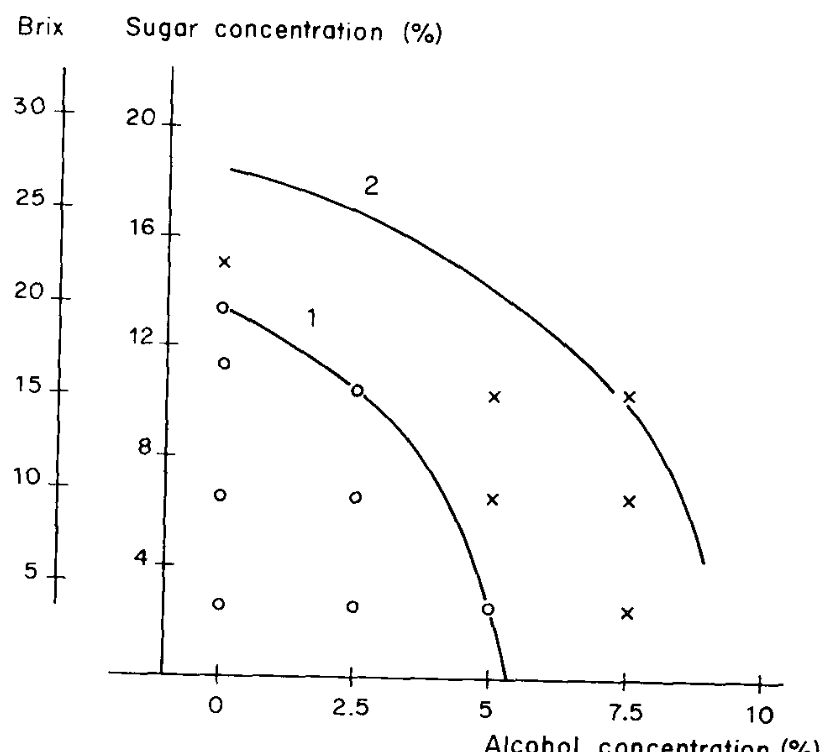

FIG. 5. — *Fermentable range of butyric acid bacteria under various alcohol and sugar concentration*

could ferment and form butyric acid. On the other hand, curve 2 shows sugar and alcohol concentration of usual rum mash during fermentation in our factory.

As apparently shown in figure 5, it was thought that this bacteria could not ferment symbiotically with rum yeast in rum mash.

II. — *Symbiotic fermentation of butyric acid bacteria and rum yeast under various sugar concentration*

Molasses media from 4.5 to 18.1 p. 100 sugar concentration were prepared, to which, each 10 p. 100 of seed culture of rum yeast and butyric acid bacteria were inoculated. After 7 days, volatile acid was determined.

As shown in figure 6, in the mash of 4.4 and 9 p. 100 sugar a large quantity of volatile acid was formed, about 80 p. 100 of the mash which was fermented by butyric acid bacteria only. In the mash of 14 and 18 p. 100 sugar, volatile acid could not be formed.

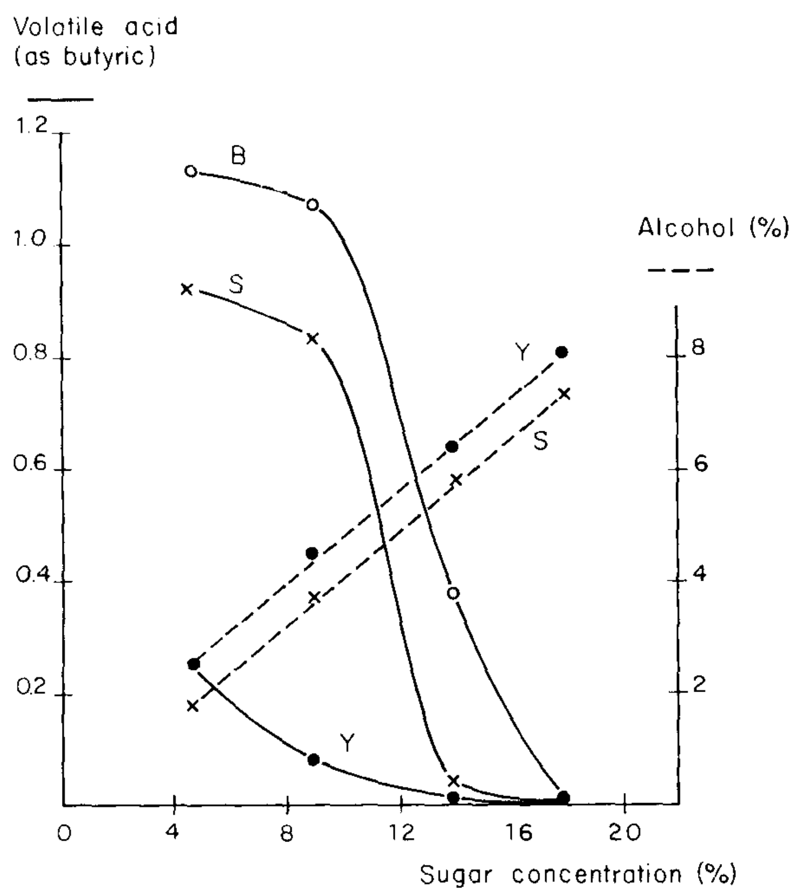

FIG. 6. — *Symbiotic fermentation of butyric acid bacteria under various sugar concentration*

As mentioned above, butyric acid bacteria could not ferment in such a high sugar medium as rum mash. Then the second method was examined : a high acid mash acidified by butyric acid fermentation was added to rum mash, and the effect on ester formation was investigated.

III. — *Ester formation from pure butyric acid by rum yeast and effect of addition of butyric acid bacteria culture to rum mash*

1. *Ester formation from pure butyric acid by rum yeast.*

At first, in order to confirm how much ester was formed from butyric acid and alcohol during fermentation by rum yeast, pure butyric acid was added to rum mash and after fermented for 7 days ester was determined. Figure 7 shows the results. Ester was a little bit increased compared with control.

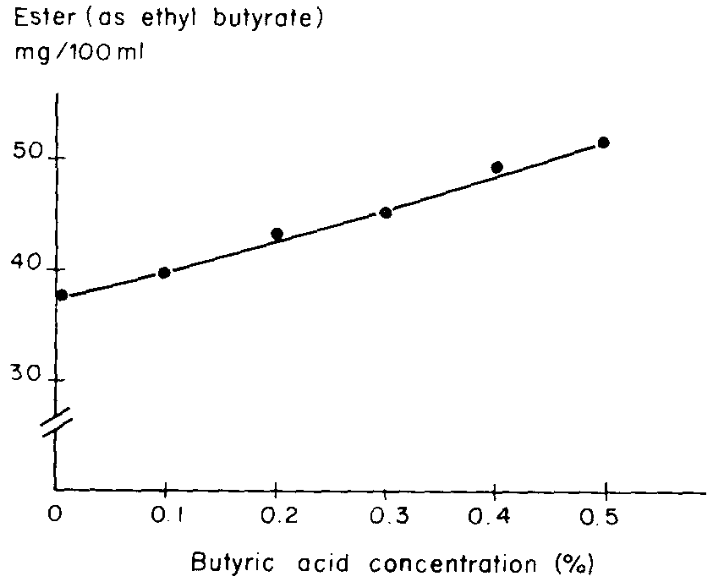

FIG. 7. — *Ester formation from pure butyric acid by rum yeast*

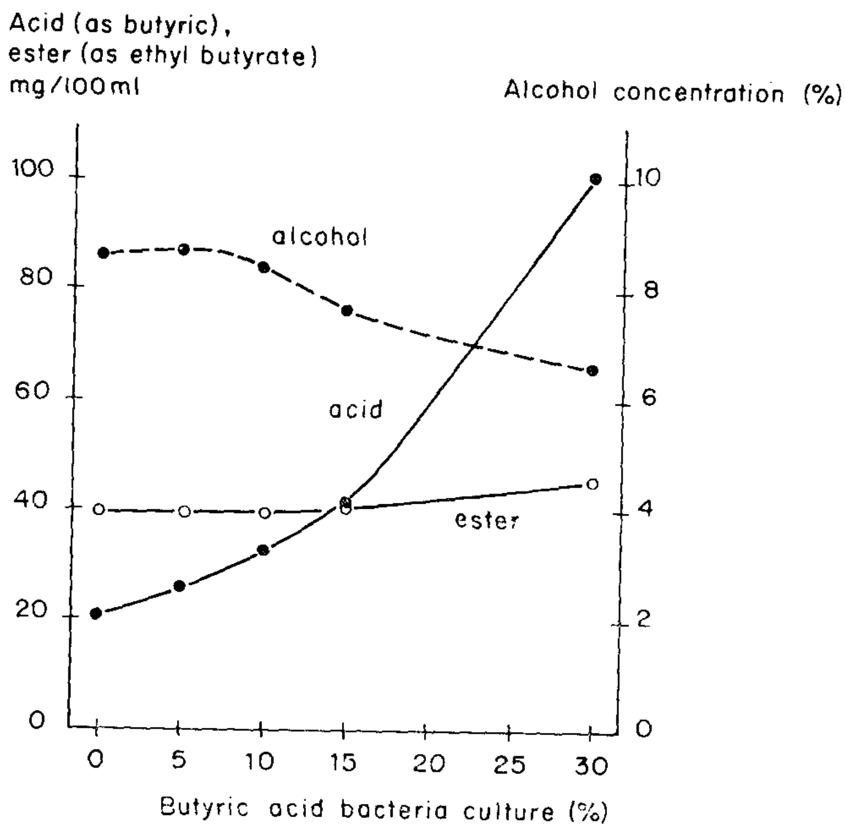

FIG. 8. *Effect of addition of butyric acid bacteria culture to rum mash*

2. *Effect of addition of butyric acid culture to rum mash.*

Five to 30 p. 100 of high acid mash was added to the rum mash to which rum yeast was simultaneously inoculated. After being fermented for 7 days, 150 ml of mash was distilled to obtain 50 ml of distillate.

As shown in figure 8, addition from 15 to 30 p. 100 of high acid mash was very effective for increasing acid content and enriching rum flavor. But ester was increased just a little as in the above experiment.

IV. — *Distillation-ratio of acetic, butyric acid, ethyl acetate, ethyl butyrate from aqueous and 10 p. 100 alcohol solution at various* Hp

The constituents of distillate might be varied largely depending on the distilling conditions, even though distilled from the same mash. Especially with volatile acid ester, pH of mash was considered to be very important factor. So, the influence of pH of mash on volatile acid and ester content of distillate was examined.

TABLE 3

Distillation ratio of acetic acid, butyric acid, ethyl acetate and ethyl butyrate from aqueous and 10 p. 100 alcohol solution at various pH

Solution to be distilled				Distilling	
		Concentration (mg/100 ml)	pH	Concentration (mg/100 ml)	Distilling ratio (%)
Aqueous solution	Acetic acid	106.2	2.0 3.5 5.0	61.2 56.4 27.0	19.2 17.7 8.4
	Butyric acid	96.8	2.0 3.5 5.0	176 176 105.6	60.6 60.6 36.3
	Ethyl acetate	50	2.0 3.5 5.0	88 86.2 88	58.7 57.5 58.7
	Ethyl butyrate	50	2.0 3.5 5.0	73.1 71.9 76.6	48.7 47.9 51.0
10 p. 100 Alcohol solution	Acetic acid	106.2	2.0 3.5 5.0	48 43.2 18.6	15 13.5 5.8
	Butyric acid	96.8	2.0 3.5 5.0	165 157 94	56.9 54.2 32.4
	Ethyl acetate	50	2.0 3.5 5.0	117 117 114	78 78 76
	Ethyl butyrate	50	2.0 3.5 5.0	111 107 107	74.2 71.1 71.1

The pH of these solutions were adjusted to 2.0-5.0, to which pure acid and ester were added separately. 150 ml of solution was distilled to obtain 50 ml of distillate. The results are shown in table 3. Volatile acid content was increased as pH of solution became, lower, while ester content was not varied.

V. — *Ester formation during distillation from 10 p. 100 alcohol solution containing acetic and butyric acid*

In order to know how much ester is formed during distillation from alcohol and acids contained in rum mash, pure acetic and butyric acid was added to 10 p. 100 alcohol solution, which was distilled at various pH. 150 ml of solution was distilled to obtain 50 ml of distillate like previous experiment.

The results are shown in table 4. Ester was formed to a large extent as pH became lower, and as acid content in solution was higher

TABLE 4

Ester formation during distillation

Solution to be distilled			Distillate	
	Concentration (mg/100 ml)	pH	Ester (mg/100 ml)	Acid (mg/100 ml)
Acetic acid	106.2	2.0	6.2	48
		3.5	4.4	43.2
		5.0	0.9	18.6
	204.2	2.0	15.8	93
		3.5	4.4	88.8
		5.0	2.6	52.8
	301.1	2.0	23.8	131.4
		3.5	5.3	131.4
		5.0	5.3	87.6
Butyric acid	96.8	2.0	5.8	165.4
		3.5	2.3	157.5
		5.0	1.1	94.2
	201.0	2.0	17.4	287
		3.5	5.8	308
		5.0	5.8	178
	302.4	2.0	18.6	442
		3.5	9.3	451
		5.0	8.1	278

VI. — *Influence of* pH *of mash on the constituent of distillate*

According to the result shown in tables 3, 4, the constituents of distillate varied largely depending on pH of solution. Then, influence of pH of mash on the constituent of distillate was examined.

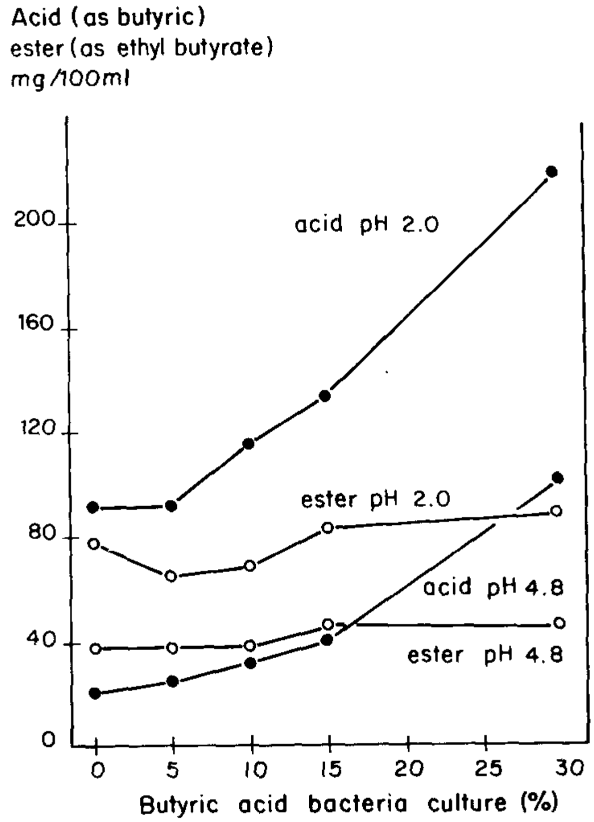

FIG. 9. — *Influence of* pH *of mash on the concentration of distillate*

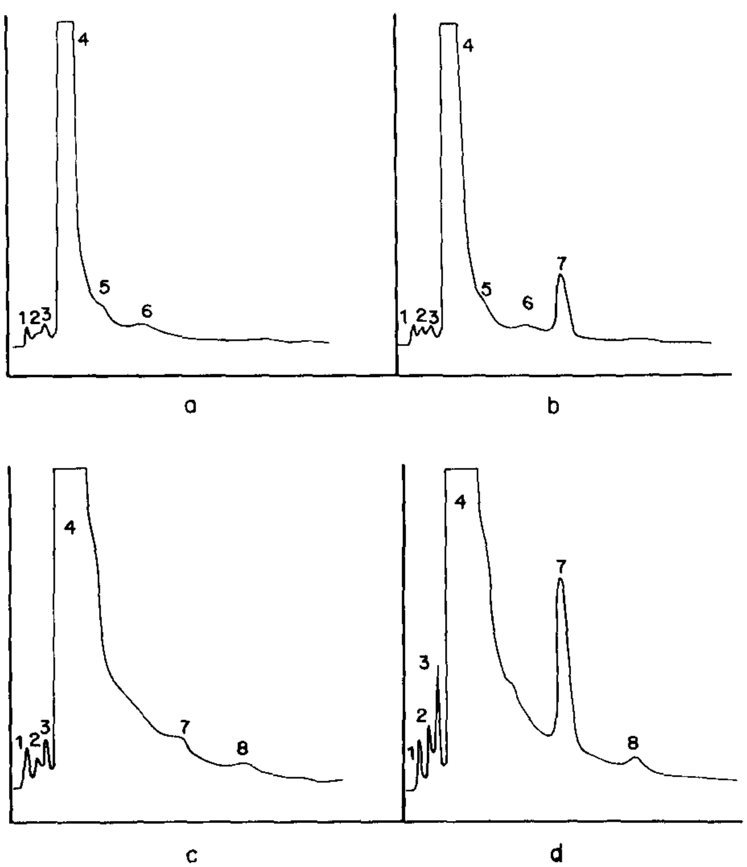

FIG. 10. — *Gas chromatogram of rum distillate*

Rum mash containing butyric acid bacteria culture 0-30 p. 100 were distillated at pH 4.8 and pH 2. The results are shown in figure 9. Not only acid was increased largely by distilled at pH 2, but ester was also increased. This might be explained as follows. By lowering pH of mash, acid was exceedingly let free which was reacted with alcohol and formed ester.

VII. — *Gas chromatogram of rum distillate*

Gas chromatograms of raw distillate from non-treated mash, raw distillate and fine distillate from pH 2 and 30 p. 100 butyric acid culture mash are shown in figure 10.

A large quantity of butanol was detected in *b* and *d*. Acetone was also increased in *b* and *d* compared with *a* and *c*. These were thought to be a result of butyric acid fermentation.

Ethyl acetate was formed in *b* and *d* more than in *a* and *c*. It might be explained that acetic acid was increased by butyric acid fermentation and largely distilled by distillating at lower pH and formed a large quantity of ester.

Besides ethyl acetate, ethyl butyrate was also thought to be formed in *b* and *d*, but it could not be detected as its peak was overlapped with ethanol. The method of preparation of sample will be further investigated.

VIII. — *Analysis of rum distillate*

Analysis of rum distillate is shown in table 5. Nº 1 is raw distillate from non-treated mash, nº 2 is raw distillate from pH 2 and 30 p. 100 butyric acid culture mash. Nº 3 and nº 4 are fine distillate of nº 1 and nº 2 respectively.

TABLE 5

Analysis of rum distillate

	Nº	1	2	3	4
	Proof	52	41.2	143.8	120.2
mg/100 ml not calculated to proof	Total acid (as acetic)	18.6	117.6	7.2	72
	Acetic acid	18.6	49.4	7.2	25.9
	Butyric acid	—	68.2	—	46.1
	Ester				
	(as ethyl acetate)	38.7	50.2	40.5	68.6
	(as ethyl butyrate)	—	66.1	—	90.5
	Aldehyde (as acetaldehyde)	16.8	20.4	19.5	23.8
	Fusel oil (as amyl alcohol)	42	20	28	61
	Furfural	1.2	3.6	4.6	5.5
	Lusson giraid number	—	—	135	384

1 : Raw distillate from non-treated mash.
2 : Raw distillate from pH 2 and 30 p. 100 B. A. culture mash.
3 : Fine distillate from the mash of 1.
4 : Fine distillate from the mash of 2.

Compared nº 4 with nº 3, total acid in nº 4 is largely increased, and about 50 p. 100 of total acid is butyric acid, while it was not detected in nº 3. Ester, fusel oil and furfural are also higher in nº 4. Aldehyde is not varied.

Lusson Giraid number is 135 in nº 3, corresponding to a light rum, while nº 4 is 384 and it indicates to be heavy type rum (JONSCHER-ZITTAU, 1974).

Tasting those distillates, flavor of fine distillate from the mash which is pH 2 and added with 30 p. 100 butyric acid culture was very much stronger than that from non-treated mash.

With the former distillate, butyric acid smelled a little bit harsh. From analytical results, the distillate seemed to be good quality as heavy rum, but it is necessary to confirm by tasting after aging for a long time if this distillate become good rum or not.

ACKNOWLEDGEMENT

The author is indebted to Dry S. SUGAMA of the Institute of Brewing for kindly offered strains of butyric acid bacteria and to Mr. N. SUGIMOTO and Mr. T. USUI for their skilled technical assistance. Thanks are due to Mr. S. NOGUCHI, *President of Gôdô Shusei and Vice-president* Mr. G. HORI, for permission to publish this work.

RÉSUMÉ

POSSIBILITÉS D'UTILISATION DE BACTÉRIES BUTYRIQUES POUR LA PRODUCTION DE RHUM

On a étudié deux méthodes de production de rhum corsé avec des bactéries butyriques.

Les bactéries butyriques ne pouvaient pas fermenter en symbiose avec la levure de rhum *Shizosaccharomyces pombe* sur un milieu à base de mélasses renfermant plus de 14 p. 100 de glucose.

L'additon de 15-30 p. 100 d'un vin fortement acide, obtenu après fermentation avec des bactéries butyriques, à un vin de rhum a permis d'accroître la teneur en acide et d'améliorer le parfum du rhum, mais l'accroissemnt de la teneur en ester a été faible.

Il a été possible de distiller le vin de rhum fermenté, renfermant du vin fortement acide, de pH bas, amené à pH 2. La teneur en acide a été multipliée par 4 à 6 et la teneur en ester par 2 par rapport à un distillat issu de vin de pH plus élevé, non ajusté. Le nombre de Lusson Giraid d'un distillat clarifié issu d'un vin non traité s'élève à 135 alors qu'il atteint 384 dans le cas d'un vin avec ajustement.

En abaissant le pH du vin, on libère l'acide butyrique, qui, pendant la distillation avec l'alcool, se combine avec ce dernier pour former des esters.

D'après les résultats analytiques, le distillat semble être d'aussi bonne qualité que le rhum corsé, mais il s'avère nécessaire de vérifier par une dégustation après une longue période de vieillissement si ce distillat se transforme ou non en bon rhum.

RESUMEN

POSIBILIDADES DE UTILIZACIÓN DE LAS BACTERÍAS BUTÍRICAS PARA LA PRODUCCIÓN DE RON

Se ha procedido al estudio de dos métodos de producción de ron fuerte, con bacterias butiricas.

Las bacterias butíricas no podían fermentar en simbiosis con la levadura del ron *Shizosac-*

charomyces pombe en un medio a base de melazas, cuyo contenido en glucosa es superior a un 14 p. 100.

La adición de un 15 a un 30 p. 100 de vino fuertemente ácido, obtenido tras fermentación con bacterias butíricas, a un vino de ron, ha permitido incrementar el contenido de ácido y mejorar el perfume del ron, pero, en cambio, el incremento del contenido en éster ha sido poco elevado.

Ha sido así posible destilar el vino de ron fermentado, que contiene vino fuertemente ácido, de pH reducido, al cual se ha dado un pH de 2. El contenido en ácido ha sido multiplicado por 4 a 6 y el contenido en éster por 2 en relación con un destilado procedente de vino de pH más elevado, no ajustado. El número de Lusson Giraid de un destilado clarificado procedente de un vino no tratado se eleva a 135, mientras que llega a alcanzar 884, en el caso de un vino con ajuste.

Al reducir el pH del vino se libera el ácido butírico, el cual — durante la destilación del alcohol — entra en combinación con este último para formar ésteres.

Según los resultados analíticos, el destilado parece ser de tan buena calidad como el ron fuerte, pero se manifiesta la necesidad de verificar, por degustación tras un largo periodo de añejamiento, si este destilado se transforma o no en ron de buena calidad.

RIASSUNTO

POSSIBILITÀ D'IMPIEGO DI BATTERI BUTIRRICI PER LA PRODUZIONE DEL RUM

Oggetto dello studio sono stati due metodi di produzione del rum generoso con dei batteri butirrici.

I batteri butirrici non potevano fermentare in simbiosi con il lievito del rum *Shizosaccharomyces pombe* su un mezzo a base di melasse che racchiudono più di 14 p. 100 de glucosio.

L'aggiunta di 15-30 p. 100 di un vino fortemente acido, ottenuto dopo fermentazione con dei batteri butirrici, a un vino di rum, ha permesso di aumentare il tenore d'acido e migliorare il profumo del rum mentre l'aumento del tenore di estere è stato debole.

E'stato possibile distillare il vino di run fermentato, rinchiudendo del vino fortemente acido, con pH basso, portato a pH 2. Il tenore in acido è stato moltiplicato per dei valori da 4 a e 6 il tenore in estere per 2 rispetto a un distillato derivato da vino con pH più elevato, non aggiustato. Il numero di Lusson Giraid di un distillato chiarificato proveniente da un vino non trattato si eleva a 135 mentre esso raggiunge 384 nel caso di un vino con aggiustamento.

Abbassando il pH del vino, si libera l'acido butirrico che, durante la distillazione con l'alcool, si combina con quest ultimo per formare degli esteri.

Secondo i risultati analitici, il distillato sembra della stessa buona qualità del rum generoso ma appare necessario degustare dopo un lungo periodo d'invecchiamento per controllare se questo distillato si trasforma o no in un buon rum.

REFERENCES

ARROYO R., 1942. *Manufacture of rum. Sugar.*, (37), 29.

JONSCHER-ZITTAU, 1974. *Zeitschr. öffentl. Chem.*, **20**, Heft 18.